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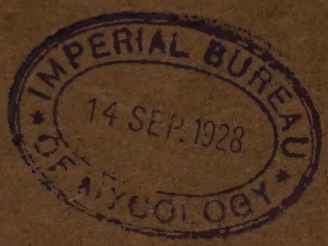

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HISTOLOGY OF THE LESIONS PRODUCED BY SPHACELOMA FAWCETTII JENKINS ON LEAVES OF CITRUS[1]

H. S. CUNNINGHAM

During the course of certain histologic investigations on diseased leaves, the attention of the writer was called to the condition existing in the leaves of *Citrus* affected with scab.

The lesions upon the leaves appear as hyperplastic areas somewhat lighter in color than that of the normal leaf, and have a corky appearance. The individual lesions are comparatively small but frequently coalesce to form a large corky patch. Where the lesions are numerous there may be considerable malformation of the affected leaf. The lesions are more commonly found on the under surface of the leaf although they do occur upon the upper surface as well.

The investigations reported in this paper were undertaken for the purpose of determining the histologic changes which take place in the infected leaf and which result in these characteristic warty excrescences. These studies include diseased leaves of three species of *Citrus*: rough lemon (*Citrus limonia* Osbeck), sour orange (*Citrus aurantium* L.), and Rangphur lime (*Citrus aurantifolia* Swingle). For purposes of comparison, wounded leaves of rough lemon were also studied.

Mature leaves bearing lesions were obtained through the courtesy of Dr. W. W. Yothers, of the Experimental Station at Orlando, Florida. This material was in excellent condition when received. Through the kindness of Dr. Anna E. Jenkins plants of rough lemon growing in the greenhouse at Cornell University were made available for wounding. The wounds were made on mature leaves by means of a common leather punch giving a circular hole two millimeters in diameter. In this way a clean cut wound was produced, care being taken to avoid cutting into or near the larger veins. The wounded leaves were removed from the tree after a period of eighteen days.

At the time of collection, or as soon thereafter as possible, small segments were cut from the leaves with a sharp razor. These segments included scab lesions or wounded tissue, as the case might be, together with

[1] This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames under whose direction the work has been done.

a portion of the healthy leaf on either side. Immediately after the segments were removed from the leaf they were placed in small shell vials containing medium chromo-acetic fixing solution (1). No vacuum pump was used to remove the air during the fixing process but the segments were kept submerged by placing a small wad of cheese cloth in the vial, thus forcing the segments below the surface of the liquid. Fixation was allowed to continue for thirty-six hours after which the material was thoroughly washed in running water and embedded in paraffin in the usual manner.

The studies were made from serial sections cut nine microns in thickness. Durand's method (2) was used in staining mycelium; crystal violet (6) for general histologic studies; and Sudan III as a test for suberin and cutin.

As the histologic changes are very similar in the diseased leaves of the three suscepts studied, it appears sufficient to present in detail the condition found in leaves of rough lemon.

REVIEW OF LITERATURE

A search of the literature revealed no satisfactory account of the histologic changes in the leaf tissue which result from attacks of the scab fungus. Numerous writers make reference to the warty appearance of the lesion and the malformation of the leaf in severe cases of infection.

Swingle and Webber (8, p. 22) state that the leaf is often considerably thickened where the wart is situated and that, as the leaves persist for at least one year, cork formation proceeds farther than in the case of the fruit. Hume (5, p. 155) says "There is often a well marked conical depression on the opposite side of the leaf corresponding to the elevation on which the excrescence is situated." Fawcett (3, p. 46) mentions the warty appearance of the scab. Grossenbacher (4, p. 134) says "When the embryonic leaves of sour-orange or grape-fruit trees begin expanding the oil-glands often protrude above the outer surface of the emerging leaves. In some cases a few of these protruding oil-glands may break open, thus giving rise to crater-like conical elevations, the upper margins of which grow more or less and tend to close the pit. In cases in which the base of such a papillum grows very strongly the former oil-gland becomes elevated on a conical growth of superficial tissues and its tip becomes covered by flaky epidermal fragments arising in what was formerly the crater-like depression of the broken oil-gland." Stevens (7, p. 84) found that as the spots enlarged they became depressed on one side and raised on the other. Winston (9, p. 12) says "Distinct hyperplasia is often in evidence beneath the area attacked by the fungus, which fact probably accounts for the plainly evident excrescence associated with the scab lesions. Specialized host tissue

can frequently be found separating invaded from uninvaded parts." This condition he found to occur in older leaves but was not observed in leaves incompletely expanded.

THE HEALTHY LEAF

In order that the reader may better understand the histologic changes which take place in the region occupied by the lesion the normal anatomy of the leaf will be presented rather fully.

In normal structure the upper epidermal cells are very regular in size and shape. They are only slightly longer than broad and are more or less rectangular in outline. The outer wall is very thick with a heavy cuticle while the radial and inner walls are also somewhat thickened. In the majority of these cells the protoplasm forms a thin peripheral layer but in others the contents are dense and granular.

The palisade parenchyma occupies approximately one half of the total thickness of the leaf, the number of cell layers varying from two to three. The cells in the two upper layers are closely packed and are considerably longer than broad while the cells of the lower layer, where such a layer exists, are shorter and broader. The cell walls are thin and the chloroplasts, while not numerous, are rather large. At intervals in the upper layer of cells and just beneath the epidermis, small cavities occur. These cavities occupy the space of two or three palisade cells and are evidently lysigenous in character.

The spongy parenchyma is comparatively open in structure and is made up of thin walled cells which are more or less oval in shape and fairly regular in outline. Each of the cells contains a large central vacuole with a layer of cytoplasm next the wall in which the chloroplasts are embedded. The cells which lie nearest the palisade layer are much larger than those lying near the lower epidermis.

The lower epidermis is similar in structure to the upper with the exception that the cells are not so thick and that some of them are more elongated. Their outer and radial walls are somewhat thickened and a relatively thin cuticle is present.

Throughout the leaf large oil-cavities are numerous. These may lie wholly within the palisade layer, wholly within the spongy parenchyma, or may occupy a portion of both of these tissues.

In the region of the larger veins only the outer layer of palisade cells is evident and these are very much shortened. The bundle proper, together with the supporting cells of thick walled parenchyma and fiber cells make up the remaining thickness of the leaf at this point.

THE DISEASED LEAF

A cross section of an infected leaf made through one of the lesions on the under surface shows a marked change from the normal at this point. This portion of the leaf is much thickened, bulging out on the lower surface with a corresponding depression on the upper surface. There has been a marked increase in the number of cells and also a marked change in the character of the cells of the tissues involved (Fig. 1).

The upper epidermal cells are apparently unchanged except that anticlinal division has taken place. The walls of the palisade cells are thickened and the chloroplasts are either reduced in number and size or have disappeared entirely. Anticlinal division has occurred and in occasional cells periclinal division as well.

The most noticeable change has taken place in the spongy parenchyma. Infection has resulted in a large increase in the number of cells as well as an increase in their size. This increase in number of cells appears to be due to division in several planes and as a result the leaf is very much thickened at this point and intercellular spaces are almost entirely wanting. In addition to the increase in number and size of cells the walls have been very greatly thickened. This thickening is entirely cellulose in nature without any indication of lignification. Many of the larger cells have been further divided by cross walls which are somewhat thinner than the main wall but of the same cellulose nature. These thick walled cells are living and each contains a peripheral layer of cytoplasm in which the nucleus is plainly visible. Early in the development of the lesion a phellogen is formed in the spongy parenchyma and as a result of the activity of this phellogen a definite phellem is laid down, thus completely isolating the portion invaded by the pathogene. The cells lying without this phellem are non-living and have suberized walls. The hyphae of the causal organism are abundant in the outer portion of this mass of suberized cells but no trace of them could be detected in the tissues lying within the phellem.

The histologic changes which take place when infection occurs on the upper surface of the leaf do not differ essentially from those already described. In such cases the phellogen is also formed in the spongy parenchyma and extends upward on either side to the upper epidermis. The phellogen thus lines a cup-like cavity filled with a mass of dead cells intermingled with hyphae. There is distinct bulging of the leaf on the under surface due to the hyperplastic condition in the spongy parenchyma.

In certain cases the phellem may extend entirely through the hyperplastic portion, thus forming a layer from the upper to the lower surface and extending as a continuous band about the edge of the lesion. Such a condition would account for the shot-hole effect sometimes accompanying this disease.

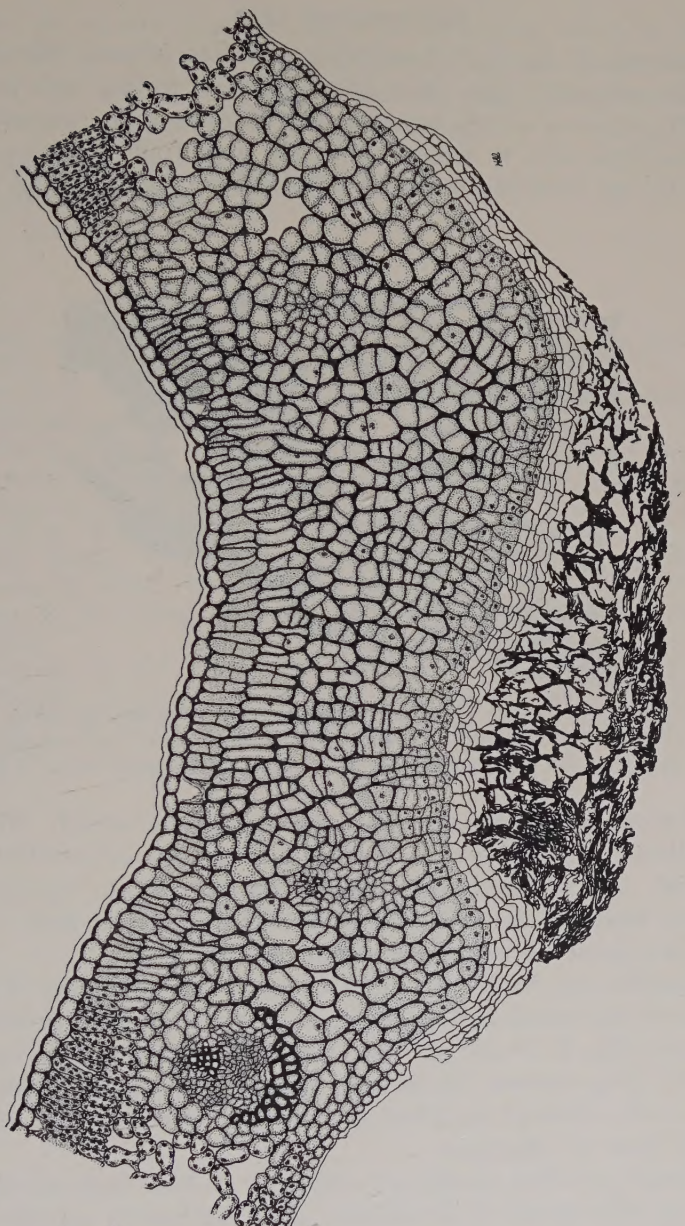

FIG. 1. A cross section through the scab lesion on a leaf of *Citrus limonia* showing the histologic changes induced by the pathogene. ×170.

THE WOUNDED LEAF

The reaction of the leaf to artificial wounding is very different from that which ordinarily takes place when infection by the scab pathogene occurs. Hyperplasia occurs, but of a very different type. A cicatrice, or band of modified cells, is formed about the wound and occupies the full depth of the leaf from one epidermis to the other (Fig. 2). This cicatrice

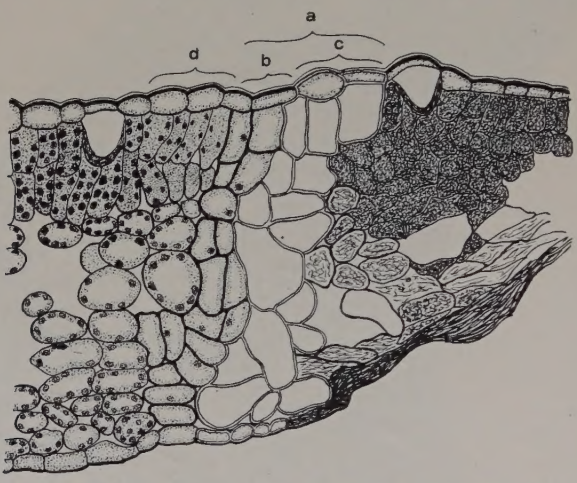

FIG. 2. Cross section through a leaf of *Citrus limonia* showing the wound periderm formed as a result of artificial wounding. a, wound periderm layer consisting of (b) phellogen and (c) phellem layers; d, modified mesophyll cells. ×300.

is formed at some little distance from the edge of the wound. The intervening cells are dead and contain a dense granular substance which resembles tannin. All of these cells, particularly those of the spongy parenchyma, are more or less collapsed, a condition which is more complete towards the edge of the wound than nearer the cicatrice.

The cicatrice may be divided into two parts: the outer part (Fig. 2, a) lying nearest the wound and consisting of a typical wound periderm with its phellogen (Fig. 2, b) and phellem (Fig. 2, c) layers; and the inner part (Fig. 2, d) lying between the wound periderm and normal tissue and in which the cells, although modified, do not show clear evidence of having originated from the phellogen.

The wound periderm consists of a layer of phellem made up of a number of large cells devoid of all contents and with heavily suberized walls. The walls of the epidermal cells adjacent to this layer are also suberized. The remainder of the wound periderm is composed of somewhat irregular shaped, thick-walled, living cells. This layer of thick-walled cells may

vary from one to several cells in width but is usually widest in the region of the spongy parenchyma. No chloroplasts are present in this layer.

In the inner layer of the cicatrice the cells vary in size, are thin-walled, and the number of chloroplasts is much reduced.

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